Platinum Complexes with Only One Purine Ligand (Guanine, Deoxyguanine, or Adenine) Flanked by Two *cis*-NH(CH_3) Groups – Informative Models for Assessing the Interaction of Purine C6 Substituents with *cis*-Amines

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Keywords: Penciclovir / Platinum / Anticancer drugs

syn-(*R*,*S*)-Me_3dienPtL complexes (Me_3dien = *N*,*N*′,*N*′′-trimethyldiethylenetriamine; L = a guanine, a 6-deoxyguanine, or an adenine derivative) represent useful models for assessing the influence of the purine C6 substituent on the rate of rotation about the Pt–N7 bond. Because of the unsymmetric nature of the *syn*-(*R*,*S*)-Me_3dien carrier ligand with respect to the coordination plane (all *N*–Me groups on one side of this plane), two rotamers are possible; they are defined as *endo* or *exo* for the six-membered ring of the purine on the same or opposite side of the coordination plane as the *N*–Me groups, respectively. The derivatives of guanine (Pen), 6-deoxyguanine (deoxy-Pen), and adenine (MeA) used are distinguished by their C6 substituent (O, H, or NH_2, respectively). At ambient temperature the rate of rotation of the purine about the Pt–N7 bond is slow on the NMR time scale for all three complexes. However, for Pen and deoxy-Pen, an increase of the temperature from 293 to 353 K led to coalescence of the ^{1}H NMR signals, indicating fast interconversion between rotamers and allowing evaluation of the rate constants and activation parameters ($\Delta H^{\neq}$ and $\Delta S^{\neq}$) for rotation. In contrast, for MeA raising the temperature to 353 K caused only a very slight broadening of the NMR signals, thus precluding a kinetic analysis of this complex by NMR methods. The rate of interconversion between the rotamers was comparable for the Pen and deoxy-Pen complexes, notwithstanding the greater bulk of the C6 substituent in the Pen complex. On the other hand, the far slower rate of rotation for MeA, as compared to Pen, cannot be explained solely on the basis of C6 substituent bulk. Activation parameters for rotation (average values for the two rotamers: $\Delta H^{\neq}$ = 57 ± 4 and 108 ± 3 kJ·mol^{-1}, $\Delta S^{\neq}$ = –18 ± 11 and 170 ± 11 J·K^{-1}·mol^{-1} for Pen and deoxy-Pen, respectively) suggest a plausible explanation for the observed trend.

Introduction

Cisplatin[1–3] and related drugs (carboplatin,[4] nedaplatin,[5] oxaliplatin[6–7]) are effective anticancer drugs widely used in the treatment of several human carcinomas. Nevertheless, limitations due to low activity toward some very common tumors (such as breast and colon carcinomas[6,8]), a variety of adverse side effects, and the development of resistance after an initial sensitivity to the treatment have fostered the search for new platinum drugs having improved therapeutic properties. There is a consensus that the antitumor efficacy of cisplatin stems from interaction with DNA and formation of intrastrand cross-links involving adjacent purines.[9–12]

Complexes of platinum with antiviral agents containing purine bases have also been investigated as potential drugs.[13–17] Although the antiviral potency of these compounds did not appear to increase with respect to that of the free base, very promising results were obtained from the viewpoint of antitumor activity. In particular, the complex *cis*-[PtCl(NH_3)$_2$(Acv)]Cl [Acv = Acyclovir = 9-(2-hydroxyethoxymethyl)guanine] proved to be active against sensitive and cisplatin-resistant tumor cells in mice.[18,19] Penciclovir {9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine, Pen}, an agent similar to Acyclovir, is also used as an antiviral drug. 6-Deoxy-Penciclovir (deoxy-Pen) is similar to Penciclovir but carries a hydrogen atom instead of the oxo group on position six of the purine. Cellular enzymes can oxidize the C6 position, introducing an oxygen atom; therefore, deoxy-Pen can be considered a pro-drug of Pen (Scheme 1).[20,21] Having available Pen, deoxy-Pen, and 9-methyladenine (MeA), we used these three bases for investigating the role of the C6 substituent in the interaction between a coordinated purine base and the *cis*-amine(s), a possible key factor affecting the formation and stability of platinum adducts with DNA.

Moreover, in order to simplify the investigation, we looked for a model system having just one purine base and symmetrical *cis*-amine groups. MedienPtL (Medien = 4-

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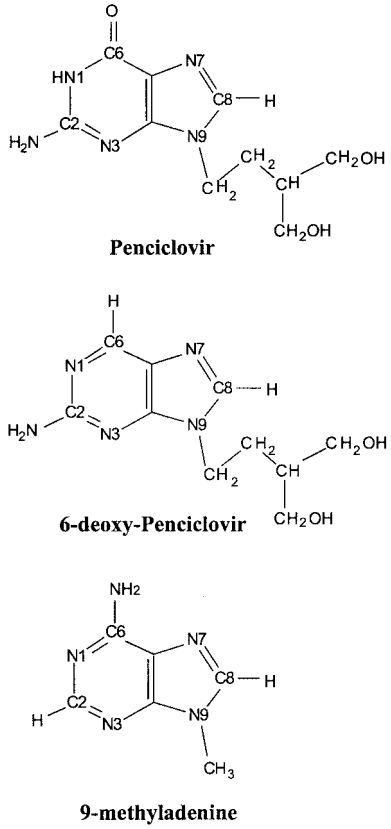
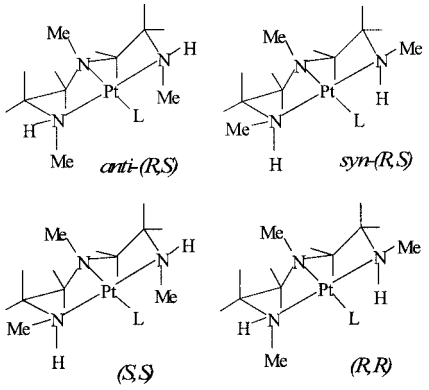
  DOI: 10.1002/ejic.200400824



Scheme 1. Schematic drawing of purine derivatives used in this study: Penciclovir (Pen), 6-deoxy-Penciclovir (deoxy-Pen), and 9-methyladenine (MeA).

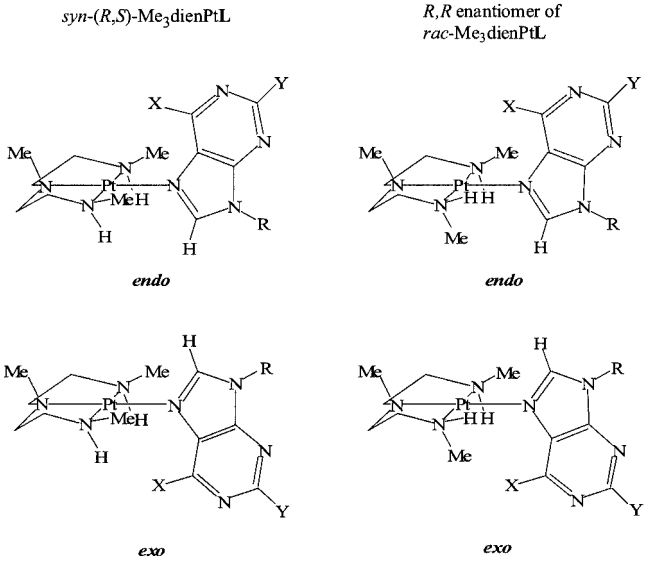
methyl-1,4,7-triazaheptane = *N*′-methyldiethylenetriamine) was the first candidate. However, the system was too dynamic for investigation by NMR (fast rotation about the Pt–N7 bond even at the lowest temperature). The rotation could be slowed by introducing methyl substituents on the terminal nitrogen atoms, and this led to the synthesis of Me_3dienPtL complexes.

The Me_3dien ligand can assume four different configurations when coordinated to the platinum atom (Scheme 2),



Scheme 2. Possible configurations for Me_3dien coordinated to platinum.

two of these are symmetrical [C_{2v} symmetry; *syn*-(*R*,*S*) and *anti*-(*R*,*S*), respectively], and two are non-symmetrical [C_s symmetry; (*R*,*R*) and (*S*,*S*), respectively].[22] [Pt(Me_3dien)-(NO_3)](NO_3) can be isolated, in the solid state, as the pure *syn*-(*R*,*S*) isomer,[23] and under acidic conditions no change of ligand configuration is observed in aqueous solution after several days at 300 K. Because of the unsymmetrical nature of the Me_3dien ligand with respect to the coordination plane, two possible rotamers are observable for restricted rotation of a coordinated purine base about the Pt–N7 bond. The two rotamers are designated as *endo* or *exo* for the six-membered ring of the purine and the central *N*–Me on the same side or on opposite sides with respect to the platinum coordination plane (Scheme 3).



Scheme 3. Possible rotamers for a coordinated purine (L) in Me_3dienPtL complexes (L = Pen, deoxy-Pen, and MeA).

In previous studies we reported the preparation and characterization by ^{1}H NMR spectroscopy of Me_3dienPtG complexes (G = guanine or inosine derivatives).[22,24] These studies established the great relevance of H-bond formation between the NH group of the carrier ligand and the 5′-phosphate of a guanine nucleotide, in comparison with a negligible interaction between an amine NH group and the guanine O6 atom. The latter interaction becomes significant only at basic pH, when deprotonation at N1 takes place.

In the present work we have chosen the same platinum substrate to investigate the effect of the substituent in position 6 of a purine base (L) upon the stability and dynamic behavior of Me_3dienPtL complexes, and we have assessed how steric, solvation, and electronic factors influence the stability and dynamic behavior.

Results

Me_3dienPtPen

The reaction of *syn*-(*R*,*S*)-[Pt(Me_3dien)(NO_3)](NO_3) with a slight excess of Pen (D_2O, pH* = 1 and 6; the asterisk denotes that no correction was made for the deuterium effect) was complete after 5 d at 298 K. At pH* = 6 (Figure 1) partial isomerization of the Me_3dien ligand from its original *syn*-(*R*,*S*) configuration to the (*S*,*S*) + (*R*,*R*) configurations (*rac*-Me_3dien) also takes place. Assignment of the resonance peaks to the *syn*-(*R*,*S*) or *rac* configurations could be performed easily by comparison with the analogous experiment performed at pH* = 1, in which case the initial *syn*-(*R*,*S*) configuration is preserved (Figure 2 for the H8 region).

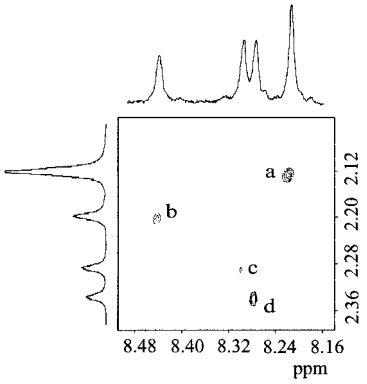


Figure 1. Selected regions of the ROESY spectrum (263 K, pH* = 6) for Me_3dienPtPen. NOE cross-peaks between purine H8 and terminal *N*–Me signals of Me_3dien are labeled. *syn*-(*R*,*S*) isomer: **a** = H8/*N*–Me for *endo* rotamer, **b** = H8/*N*–Me for *exo* rotamer. *rac* Isomer: **c** = H8/*syn*-*N*–Me for *exo* rotamer, **d** = H8/*anti*-*N*–Me for *endo* rotamer.

For both the *syn*-(*R*,*S*) and *rac* isomers two rotamers are possible (Scheme 3). The *endo* rotamer has the six-membered ring of the purine on the same side as the central *N*–Me group of Me_3dien with respect to the platinum coordination plane. In contrast, the *exo* rotamer has the six-membered ring of the purine on the opposite side of the central *N*–Me group with respect to the coordination plane. At 263 K (20% CD_3OD added to the D_2O solution) the exchange between rotamers is slow on the NMR time scale for both isomers; therefore, two relatively sharp and well-separated H8 signals are observed for each isomer (Figure 1, Table 1).

syn-(*R*,*S*)-Me_3dienPtPen has one major and one minor rotamer. In the ROESY spectrum (Figure 1) the H8/*N*–Me-(terminal) cross-peak for the major rotamer (a) was twice the size of that for the minor rotamer (b).

The H8/*N*–Me distance is expected to be shorter in the *exo* rotamer (H8 near the *N*–Me groups) than in the *endo* rotamer; however, in the present case the size of each cross-peak is proportional to the intensity of the parent resonances and therefore cannot be diagnostic for the *endo* or *exo* conformation of a given rotamer. However, for *syn*-(*R*,*S*)-Me_3dienPtG complexes, extensively investigated in a previous work, it was demonstrated that the H8 resonance at higher field (the most intense) belongs to the *endo* rotamer while the resonance at lower field (the less intense) belongs to the *exo* rotamer.[22,24] Thus, on this basis, the major rotamer has the *endo* conformation.

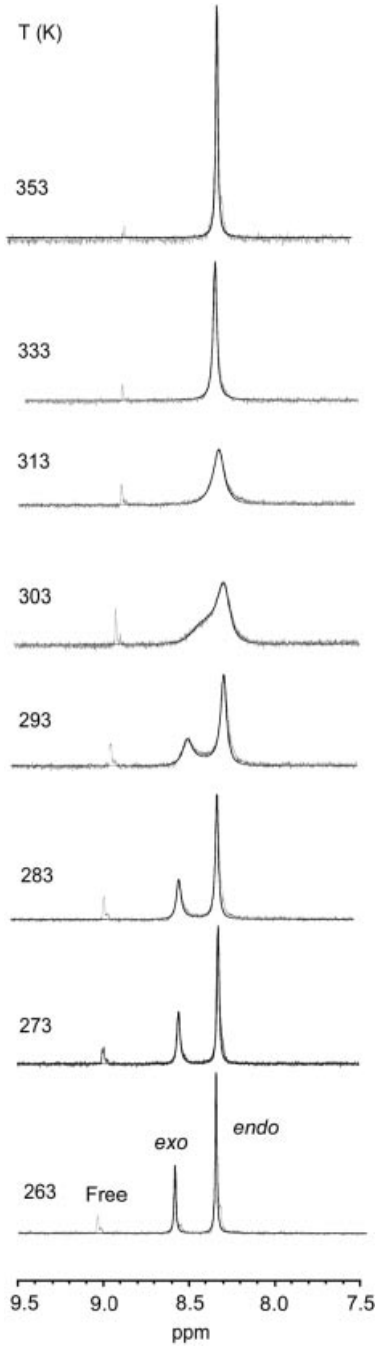


Figure 2. Experimental (pH* = 1, gray line) and simulated (dark line) NMR spectra in the H8 region for *syn*-(*R*,*S*)-Me_3dienPtPen at sample temperatures indicated.

For the adduct with the *rac* configuration of Me_3dien, the two rotamers were formed in equal amounts (equal intensity of the H8 signals). *rac*-Me_3dien has one terminal *N*–Me on the same side of the coordination plane (*syn*-*N*–Me) and the other on the opposite side (*anti*-*N*–Me) as the cen-

Table 1. ^{1}H NMR shifts (ppm) and distribution ratios for Me_3dienPtL complexes (L = Pen, deoxy-Pen, and MeA).

L	Rotamer	H6/H2	H8	Central *N*–CH_3	*anti* *N*–CH_3	*syn* *N*–CH_3	*endo*/*exo* ratio
Pen[a]			7.74				
syn-(*R*,*S*)	*endo*		8.22	3.06		2.13	2.3
	exo		8.45	3.02		2.20	
rac	*endo*		8.28	3.00	2.34	2.29	1
	exo		8.30	3.00	2.34	2.29	
deoxy-Pen[b]		8.52	8.09				
syn-(*R*,*S*)	*endo*	9.10	8.58	3.10		2.17	1.4
	exo	8.91	8.83	3.08		2.18	
rac	*endo*	9.01	8.67	3.05	2.37	2.10	1.4
	exo	8.96	8.73	3.01	2.37	2.08	
MeA[c]		8.45	8.31				
syn-(*R*,*S*)	*endo*	8.62	9.03	3.24		2.34	2.9
	exo	8.62	9.30	3.21		2.33	
rac	*endo*	8.62	9.11	3.22	2.54	2.28	2.2
	exo	8.62	9.22	3.16	2.51	2.28	

[a] pH* = 6, 263 K, proton signal of HOD set at δ = 5.1 ppm. [b] pH* = 5.3, 258 K, proton signal of HOD set at δ = 5.2 ppm. [c] pH* = 1, 278 K, proton signal of HOD set at δ = 5.0 ppm.

tral *N*–Me. An NOE cross-peak between the central *N*–Me and one terminal *N*–Me (δ = 2.29 ppm) assigns the latter signal to the *syn*-*N*–Me; the remaining terminal *N*–Me signal at δ = 2.34 ppm is consequently assigned to the *anti*-*N*–Me. The *endo* rotamer is expected to give an H8/*anti*-*N*–Me NOE cross-peak (both groups on the same side of the coordination plane), while the *exo* rotamer is expected to give a cross-peak between the H8 and the *syn*-*N*–Me signals. The H8 signal at δ = 8.30 ppm has a cross-peak with the *syn*-*N*–Me signal at δ = 2.29 ppm (c in Figure 1), while the H8 signal at δ = 8.28 ppm has a cross-peak with the *anti*-*N*–Me signal at δ = 2.34 ppm (d in Figure 1). Therefore, the signal at δ = 8.30 ppm is assigned to H8 of the *exo* rotamer and that at δ = 8.28 ppm to H8 of the *endo* rotamer.

Cross-peak d is considerably more intense than cross-peak c (Figure 1), indicating that when the six-membered ring of the guanine is adjacent to the "quasi equatorial" *syn*-*N*–Me (*endo* rotamer), the H8 atom can come close to the "quasi axial" *anti*-*N*–Me. In contrast, when the six-membered ring of the guanine is adjacent to the "quasi axial" *anti*-*N*–Me (*exo* rotamer), the H8 atom is kept at some distance from the "quasi equatorial" *syn*-*N*–Me. This finding is in line with a recent observation indicating that there is greater steric interaction between O6 (of an N7-coordinated guanine) and a "quasi axial" *N*–Me than between O6 and a "quasi equatorial" *N*–Me.[25]

In *syn*-(*R*,*S*)-Me_3dienPtPen, rate constants and activation parameters for interconversion between rotamers were evaluated by simulation of ^{1}H NMR spectra recorded at pH* = 1 and at various temperatures between 258 and 353 K (Figure 2, Table 2). The enthalpy and entropy of activation ($\Delta H^{\neq}$ and $\Delta S^{\neq}$, respectively) were estimated from plots of $\ln(k/T)$ vs. $1/T$, which were linear within experimental error (Supporting information; for Supporting information see also the footnote on the first page of this article, Table 4). With respect to the transition state, the *endo* rotamer is lower in enthalpy by 61 ± 2 kJ·mol^{-1} and slightly higher in entropy by 8 ± 2 J·K^{-1}·mol^{-1}. Also the *exo* rotamer is lower in enthalpy by 54 ± 2 kJ·mol^{-1} but significantly higher in entropy by 29 ± 2 J·K^{-1}·mol^{-1}.

From the activation parameters, it is possible to calculate the difference in enthalpy (ΔH) and entropy (ΔS) between *endo* and *exo* rotamers. Compared to the *exo* rotamer, the *endo* rotamer is lower in both enthalpy and entropy (by 7 ± 2 kJ·mol^{-1} and 21 ± 2 J· K^{-1}·mol^{-1}, respectively).

Me_3dienPtdeoxy-Pen

The reaction of *syn*-(*R*,*S*)-[Pt(Me_3dien)(NO_3)](NO_3) with deoxy-Pen in D_2O solution at 298 K was performed at pH* = 1 and 6 for 5 d. In the experiment performed at the higher pH partial isomerization of the Me_3dien ligand from its original *syn*-(*R*,*S*) configuration to the *rac* configuration was observed. Assignment of the resonance peaks to the *syn*-(*R*,*S*) and *rac* isomers was facilitated by comparison with the results of the analogous experiment performed at pH* = 1, for which no isomerization of the *syn*-(*R*,*S*) ligand configuration took place (Figure 4). In order to distinguish between the H6 and H8 set of signals, an aliquot of the NMR samples was heated at 70 °C for several hours to allow H8 exchange with D_2O. The signals that disappeared were assigned as H8 signals.

Coordination of deoxy-Pen took place only through N7 at both pH* values. This conclusion is supported by several pieces of evidence such as: i) formation of the platinum adduct under conditions (pH* = 1) in which the N1 atom is fully protonated ($pK_a \approx 4.3$ as determined by NMR experiments); ii) upfield shift (ca. 0.25 ppm) of the H6 signal (but not of the H8 signal, which instead remained constant) as the pH* was raised from 1 to 6 (reflecting deprotonation at N1); iii) coalescence of the two H6 signals and of the two H8 signals of each isomer [only the *syn*-(*R*,*S*) isomer for the experiment performed at pH* = 1; the *syn*-(*R*,*S*) and the *rac* isomers for the experiment performed at pH* = 6] as the temperature was raised from 255 to 353 K. Coalescence could not be possible if the two H6 signals or the two H8 signals originated from isomers with a different mode of coordination of deoxy-Pen (N7- and N1-coordination).

The coordination, at the same pH* = 6 (where N1 is not protonated), of deoxy-Pen exclusively through N7 and of MeA through both N7 and N1 can be easily understood if we consider the position of the NH_2 substituent on the six-membered ring. In deoxy-Pen the NH_2 substituent is on position 2 of the ring and it can inhibit Pt binding at N1 but not N7; thus N7 binding is preferred. In MeA, in contrast, the NH_2 substituent is on position 6 and it can inhibit Pt binding at both N1 and N7. Thus, binding at both sites is still competitive, and two isomers that cannot interchange rapidly are formed.

The assignment of proton signals to the *endo* and *exo* conformers of each isomer [*syn*-(*R*,*S*) and *rac*] has been achieved by a ROESY experiment carried out at pH* = 5.3 and 258 K (30% CD_3OD added to the D_2O solution); the

Table 2. Rate constants and percentages of rotamers used to simulate NMR spectra (pH* = 1) of *syn*-(*R*,*S*)-Me_3dienPtPen at different temperatures.

T [K]	Rate constants [s^{-1}]		Percentages of rotamers	
	k_1 (*endo* → *exo*)	k_{-1} (*exo* → *endo*)	*endo*	*exo*
258	–	–	71.20	28.80
263	–	–	69.87	30.13
268	2.00	4.42	68.18	31.82
273	3.80	7.69	66.93	33.07
278	6.00	11.61	65.93	34.07
283	9.50	17.18	64.39	35.61
288	15.50	26.60	63.18	36.82
293	28.00	45.40	61.85	38.15
298	39.85	61.56	60.71	39.29
303	66.70	97.00	59.25	40.75
308	102.00	142.60	58.30	41.70
313	137.00	181.00	56.92	43.08
323	268.00	323.00	54.65	45.35
333	614.00	672.00	52.26	47.74
343	1140.00	1164.00	50.52	49.48
353	2230.00	2240.00	50.11	49.89

low temperature ensures the observation of relatively sharp signals (Figure 3). The most upfield signals in the aliphatic region (δ = 2.0–2.2 ppm) are assigned to the terminal *syn*-*N*–Me signals, all of them having NOE cross-peaks with the central *N*–Me signals falling in the δ = 3.0–3.2 ppm region (Supporting information). The signals at δ = 2.17 and 2.18 ppm (one signal for each rotamer) belong to the *syn*-(*R*,*S*) isomer, while the signals at δ = 2.08 and 2.10 ppm (one signal for each rotamer) belong to the *syn*-*N*–Me of the *rac* isomer. The *anti*-*N*–Me signals of the two rotamers of *rac*-Me_3dienPtdeoxy-Pen overlap at δ = 2.37 ppm. Terminal *N*–Me signals were used to assign the signals of the aromatic protons. The a,b and a′,b′ pairs of resonances in the 1D trace (Figure 3) belonging to the *syn*-(*R*,*S*) isomer are assigned to the H8 and H6 protons, respectively, because of the presence of EXSY cross-peaks between a and b and between a′ and b′ resonances (not shown). The more intense pair of signals (a,a′) is characterized by having a stronger NOE between the H6 and terminal *N*–Me signals (cross-peak a′ in the 2D spectrum) than between the H8 and terminal *N*–Me signals (cross-peak a); therefore, this intense pair is assigned to the *endo* rotamer (H6 adjacent to *N*–Me groups). The weaker pair of signals (b,b′) is thus assigned to the *exo* rotamer. The *endo*/*exo* ratio is ca. 1.4.

For the *rac* isomer, again two rotamers of slightly different abundances are observed (pairs of H8,H6 signals, c,c′ and d,d′). For the more intense pair of signals (c,c′) the H8 signal (c) has a cross-peak to the *anti*-*N*–Me signal, and the H6 signal (c′) has a cross-peak to the *syn*-*N*–Me signal; thus, c,c′ are assigned to the *endo* rotamer. In contrast, for the weaker pair of signals (d,d′) the H8 signal (d) has a cross-peak to the *syn*-*N*–Me signal, and the H6 signal (d′) has a cross-peak to the *anti*-*N*–Me signal; therefore d,d′ are assigned to the *exo* rotamer.

Within the set of H8 signals for the two isomers (Figure 3), the most upfield signals belong to *endo* rotamers, while the most downfield signals belong to *exo* rotamers, as found previously.[24] In contrast, the H6 signals show the reverse trend (the most upfield signals belong to the *exo* rotamers, while the most downfield signals belong to the *endo* rotamers).

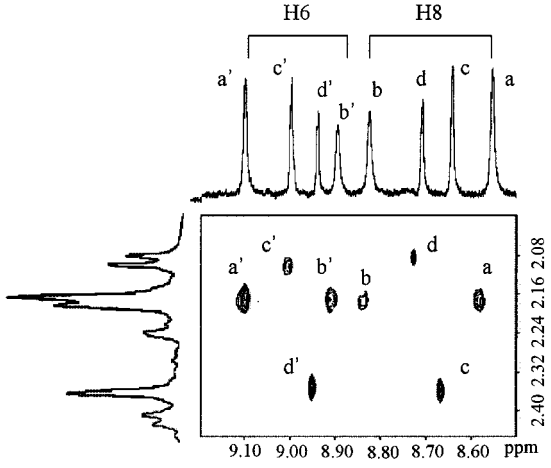


Figure 3. Selected regions of the ROESY spectrum (258 K, pH* = 5.3) for Me_3dienPtdeoxy-Pen. Protons H8 (a−d) and H6 (a′−d′) are labeled.

For *syn*-(*R*,*S*)-Me_3dienPtdeoxy-Pen, the rate constants for interconversion between rotamers were evaluated by spectral simulation between 255 K and 353 K performed at pH* = 1 (Figure 4, Table 3).

It is noteworthy that for the *exo* rotamer the chemical shifts of the H8 and H6 signals are rather close. One-dimensional NMR experiments show that a change of pH* from 1 (protonated N1) to 5.3 (non-protonated N1) causes a change in the relative positions of these two resonances (the H8 signal downfield to the H6 signal at pH* = 1 and upfield at pH* = 5.3). ROESY experiments performed at the two pH* values confirmed the correctness of all 1D assignments.

The enthalpy and entropy of activation ($\Delta H^{\neq}$ and $\Delta S^{\neq}$, respectively) were estimated from plots of ln(k/T) vs. 1/T, which were linear within experimental error (Supporting information, Table 4). With respect to the transition state, both the *endo* and *exo* rotamers are lower in enthalpy (by

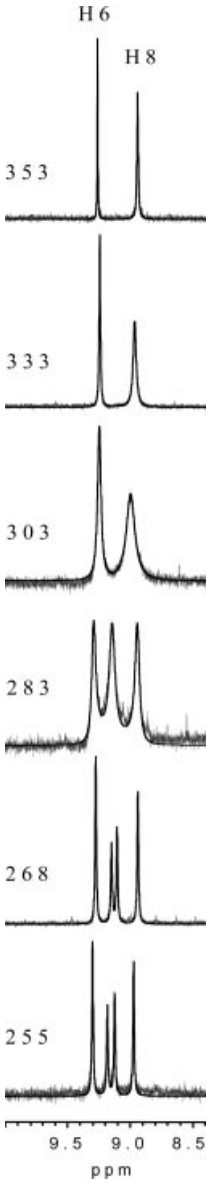


Figure 4. Experimental (pH* = 1, gray line) and simulated (dark line) NMR spectra in the H8 region for *syn*-(*R*,*S*)-Me_3dienPtdeoxyPen at sample temperatures indicated.

111 ± 2 and 105 ± 2 kJ·mol^{-1}, respectively) and entropy (by 181 ± 4 and 159 ± 4 J·K^{-1}·mol^{-1}, respectively). The *endo* rotamer is lower in enthalpy (6 kJ·mol^{-1}) and entropy (22 J·K^{-1}·mol^{-1}) than the *exo* rotamer. An analogous trend was already noted for the Pen complex (ΔH of 7 kJ·mol^{-1} and ΔS of 21 J·K^{-1}·mol^{-1}).

Me_3dienPtMeA

The reaction of *syn*-(*R*,*S*)-[Pt(Me_3dien)(NO_3)](NO_3) with a slight excess of MeA (molar ratio 1:1.4) was carried out in D_2O, pH′ = 1. The low pH ensures not only the absence of isomerization of the carrier ligand from its original *syn*-(*R*,*S*) configuration, but also coordination of adenine through N7 because N1 is fully protonated (pK_a = 4.3).[26] After 3 d at 298 K, 80% of the initial platinum complex had reacted, and another aliquot of MeA was added (Figure 5).

On the basis of their different intensities, the resonances can be grouped into two sets. The most intense set includes signals at δ = 3.82, 8.53, and 8.94 ppm; the second set, less intense, includes signals at δ = 3.84, 8.51, and 9.19 ppm. The intensity ratio between the two sets is ca. 3. The presence of two sets of resonances can be explained by the presence of two rotamers.

For each set of resonances the intensity of the most downfield signal decreased with time (δ = 8.94 and 9.19 ppm, respectively). Such a decrease in intensity is expected to take place for signals belonging to H8 protons, which are known to undergo exchange with deuterium of the solvent.[27,28] The downfield shift of the H8 and H2 resonances, consequent to coordination to the platinum atom (ca. 0.7 and 0.15 ppm, respectively), and the differences in chemical shift between the two H8 ($\Delta\delta$ = 0.25 ppm) and the two H2 resonances ($\Delta\delta$ = 0.02 ppm) are in agreement with N7 coordination of MeA. In fact, the H8 proton, being much closer to the donor atom (N7) than H2, will not only be more deshielded by the metal center, but will also experience more different chemical environments in the two rotamers.

The correct assignment of the H8 and H2 resonances was also confirmed by an inversion recovery experiment (Supporting information): the longitudinal relaxation time (T_1) of the H2 protons was about three times longer than that of the H8 protons, as previously reported for adenine derivatives.[29]

Although the coordinated *syn*-(*R*,*S*)-Me_3dien configuration is rather stable at low pH, a sample of Me_3dienPtMeA kept at 298 K for 30 d showed partial isomerization from the initial *syn*-(*R*,*S*)-Me_3dienPtMeA to the *rac*-Me_3dienPtMeA. A NOESY experiment was performed on this sample (pH* = 1, 278 K) in order to assign the *endo* or *exo* conformation to the two rotamers of the *syn*-(*R*,*S*) and *rac* species.

NOE cross-peaks between central and terminal *N*–Me signals (Figure 6 A) allow us to distinguish between *syn*- and *anti*-*N*–Me groups. For *syn*-(*R*,*S*)-Me_3dienPtMeA the NOE cross-peaks between central and terminal *N*–Me resonances (labeled a) fall at δ = 2.33/3.21 ppm (for the minor rotamer) and at δ = 2.34/3.24 ppm (for the major rotamer). For the *rac* isomer, the terminal *N*–Me signal at δ = 2.28 ppm gives an NOE cross-peak with the central *N*–Me signal at δ = 3.16 ppm (coincident peaks for the two rotamers, b); therefore, the resonance at δ = 2.28 ppm is assigned to the *syn*-*N*–Me group of both rotamers. As a consequence, the resonances of the terminal *N*–Me groups at δ = 2.51 and 2.54 ppm (two distinct signals for the two rotamers), which do not give NOE cross-peaks with the central *N*–Me resonances, are assigned to the *anti*-*N*–Me signals of the minor and major rotamer of the *rac* isomer, respectively.

For *syn*-(*R*,*S*)-Me_3dienPtMeA the minor rotamer has an intense NOE cross-peak between H8 and the terminal *N*–Me signals (δ = 9.30/2.33 ppm, c in Figure 6 B). In contrast, the major rotamer (H8; δ = 9.03 ppm) lacks such a cross-

Table 3. Rate constants and percentages of rotamers used to simulate NMR spectra (pH* = 1) of *syn*-(*R*,*S*)-Me_3dienPtdeoxy-Pen at different temperatures.

T [K]	Rate constants [s^{-1}]		Percentages of rotamers	
	k_1 (*endo* → *exo*)	k_{-1} (*exo* → *endo*)	*endo*	*exo*
255	–	–	59.80	40.20
258	0.55	0.76	58.20	41.80
263	1.32	1.83	58.00	42.00
268	3.53	4.53	57.50	42.50
273	10.17	13.19	56.50	43.50
278	21.92	25.73	54.00	46.00
283	53.27	58.88	52.60	47.40
288	123.09	130.71	51.50	48.50
293	278.71	281.51	50.20	49.80
298	612.22	594.13	49.30	50.70
303	1317.82	1204.39	47.80	52.20
313	5631.90	4751.44	45.90	54.10
323	21974.81	17114.00	43.80	56.20
333	79074.24	57419.69	42.10	57.90
343	265065.02	177678.40	40.20	59.80
353	827751.32	522545.87	38.80	61.20

Table 4. Activation enthalpy [kJ·mol^{-1}] and entropy [J·K^{-1}·mol^{-1}] for interconversion between rotamers in *syn*-(*R*,*S*)-Me_3dienPtL, at pH′ = 1.

L	$\Delta H^{\neq}_{endo}$	$\Delta H^{\neq}_{exo}$	$\Delta S^{\neq}_{endo}$	$\Delta S^{\neq}_{exo}$
Pen	61 ± 2	54 ± 2	–8 ± 2	–29 ± 2
EtG[24]	65 ± 2	56 ± 2	2 ± 2	–28 ± 2
deoxy-Pen	111 ± 2	105 ± 2	181 ± 4	159 ± 4

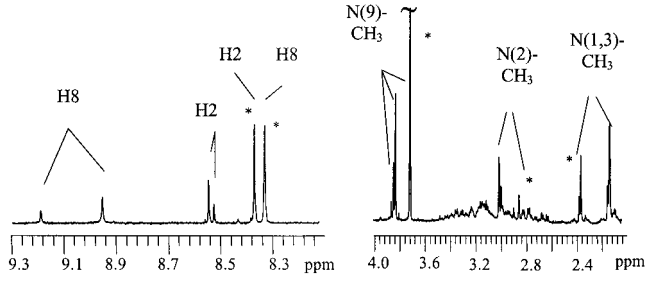


Figure 5. ^{1}H NMR spectrum (295 K, pH* = 1) of *syn*-(*R*,*S*)-Me_3dienPtMeA in the presence of an excess of MeA and some unchanged *syn*-(*R*,*S*)-$[Pt(Me_3dien)(H_2O)]^{2+}$ substrate (signals labeled with an asterisk).

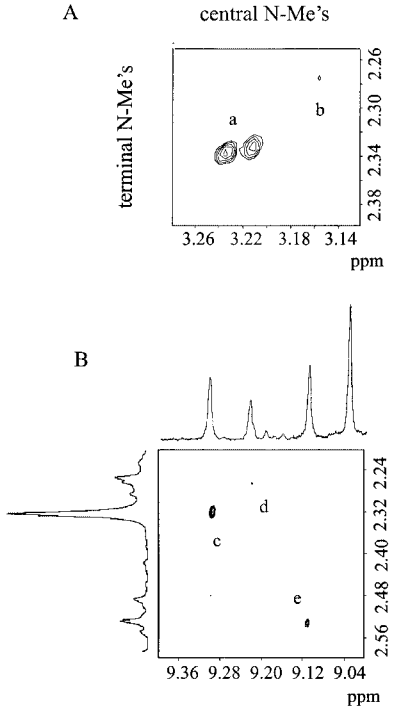


Figure 6. Selected regions of the NOESY spectrum (278 K, pH* = 1) of Me_3dienPtMeA. Spectrum A: Equatorial *N*–Me/central *N*–Me region; NOE cross-peaks are labeled a and b for the *syn*-(*R*,*S*) and *rac*-Me_3dienPtMeA, respectively. Spectrum B: Terminal *N*–Me/aromatic proton region; NOE cross-peaks are labeled c, d, and e for the *exo* rotamer of *syn*-(*R*,*S*)-Me_3dienPtMeA and for the *exo* and *endo* rotamers of the *rac* isomer, respectively.

peak. Thus, it is possible to assign the *exo* conformation to the minor rotamer (H8 near the terminal *N*–Me group with respect to the coordination plane) and the *endo* conformation to the major rotamer.

For *rac*-Me_3dienPtMeA, NOE cross-peaks were observed between the H8 signal of the minor rotamer and the *syn*-*N*–Me signal (δ = 9.22/2.28 ppm, d in Figure 6 B), and between the H8 signal of the major rotamer and one *anti*-*N*–Me signal (δ = 9.11/2.54 ppm, e in Figure 6 B); therefore, the *exo* form is assigned to the minor rotamer and the *endo* form to the major rotamer.

For *syn*-(*R*,*S*)-Me_3dienPtMeA, ^{1}H NMR spectra recorded between 268 and 353 K did not show significant broadening of the resonance peaks, indicating that the rotation around the Pt–N7 bond is slow on the NMR time scale, even at 353 K.

Discussion

In a previous study the Me_3dienPtL system (L = 9-ethylguanine, 5′-guanosine monophosphate, and 3′-guanosine monophosphate; EtG, 5′-GMP, and 3′-GMP, respectively) was used to investigate the effect of the substituent in position 9 of a guanine base upon the rotamer ratio and the

rate of rotamer interconversion. The most interesting result of that investigation was the great tendency of the 5′-phosphate to form an H bond to the NH group of a *cis*-amine when phosphate and N–H are on the same side of the platinum coordination plane. As a consequence, the *endo* rotamer was the exclusive form for the *syn*-(*R*,*S*) species (at neutral and basic pH). In contrast, for the EtG and 3′-GMP complexes, in which such an interaction cannot take place, the *endo* and *exo* rotamers were present in comparable amounts with a slight preference for the *endo* rotamer. Moreover, in one case (EtG complex) the rate of interconversion between *endo* and *exo* rotamers was investigated by ^{1}H NMR and the activation parameters ($\Delta H^{\neq}$ and $\Delta S^{\neq}$) evaluated. Because the rate of rotation is expected to be very much dependent upon the substituent in position 6 of the ring system, we looked for purine bases differing in the nature of this substituent. Penciclovir, deoxy-Penciclovir, and 9-methyladenine appeared to be most appropriate because two of them have moderately bulky hydrophilic substituents of opposite surface charge (electron-rich oxygen in Penciclovir and electron-poor amine protons in adenine), and deoxy-Penciclovir has a sterically much less demanding H substituent.

Factors Influencing Rotation Around the Pt–N(7) Bond

Most notably, the behavior of the Pen derivative is very similar to that of the EtG derivative previously investigated.[24] The Pen and EtG complexes have not only very similar ratios between *endo* and *exo* rotamers, but also very similar rate constants and activation parameters for interconversion (Table 4). This finding indicates that the different nature of the N9 substituents [Et and 4-hydroxy-3-(hydroxymethyl)butyl for EtG and Pen, respectively] does not influence the ratio and interconversion rate of rotamers. The N9 substituents project in a direction opposite to that of the platinum core, and there is little chance for interaction with the *cis*-amine groups [except in the case of 5′-phosphate-(deoxy)ribo derivatives].

Quite surprising are the comparable rates of rotation observed in the compounds with Pen and deoxy-Pen. We expected a much faster rate of rotation in the deoxy-Pen derivative because of the much smaller steric bulk of the proton, as compared to an oxygen atom, at position 6 of the purine system. A possible explanation for this apparent paradox is suggested by the activation parameters for rotation about the Pt–N7 bond. In the deoxy-Pen complex the transition state is much higher in both enthalpy (average 108 ± 3 kJ·mol^{-1}) and entropy (average 170 ± 11 J·K^{-1}·mol^{-1}), with respect to the *endo* and *exo* ground states, than in the Pen complex (average $\Delta H^{\neq}$ and $\Delta S^{\neq}$ of 57 ± 4 kJ·mol^{-1} and -18 ± 11 J·K^{-1}·mol^{-1}, respectively). It is therefore rather surprising that in the case of Pen, carrying a rather bulky substituent in position 6 of the purine ring (O), the enthalpy of activation is ca. 50 kJ·mol^{-1} lower than in the case of deoxy-Pen, carrying a much smaller group (H).

We believe that the charge of the C6 substituent, in addition to its steric bulk, is very important also. It has been observed that the H8 of a purine carries a partial positive charge and also H6 (in the case of deoxy-Pen), although to a lesser extent, is likely to carry a partial positive charge.[30] In contrast, the O6 of Pen is electron rich. In the transition state, the purine is dragged through the platinum coordination plane, and H8 and X6 (X = O or H) approach the *cis*-amines. Because of the electronegativity of the nitrogen atom, the amine H and Me groups are expected to bear a partial positive charge. For Pen the opposite charge of O6 and of the *cis*-amine substituents (H or Me) evidently favors the bulky O6 group passing through the coordination plane. In other words, the O6 of Pen is an H bond acceptor while the amine group is an H-bond donor, and the attractive interaction between the two groups is likely to lower the rotational barrier. Pen also has very little entropy of activation. If, as we believe for an intramolecular process, the entropy of activation is mainly related to solvation/desolvation phenomena, we must conclude that in the present case (attractive interaction between the two groups passing by) there is only a reorganization of the solvation shells, without release of water molecules.

For deoxy-Pen, the purine H6 and the *cis*-amine substituents (H or Me) both carry a partial positive charge. The electrostatic repulsion between the two positively charged moieties, coming close to one another, will increase the rotational barrier, notwithstanding the small size of H6 compared to O6 of Pen. Moreover, deoxy-Pen (unlike Pen) has a very large entropy of activation (average 170 ± 11 J·K^{-1}·mol^{-1}). We propose that, in the presence of an electrostatic repulsion between the two groups passing by, there is a large desolvation process with consequent release of water molecules. This desolvation process will result in a large activation entropy and a further increase of activation enthalpy.

The explanation given above for the Pen and deoxy-Pen complexes fully accounts for the behavior of the MeA derivative, in which the substituent in position 6 of the purine ring is bulky (even more than O6 of Pen) and positively charged (like the substituents on the *cis* amine). Under these circumstances the barrier to rotation is expected to be far greater than that observed for Pen or deoxy-Pen, with the consequence that the interconversion between rotamers remains slow on the NMR time scale, even at the highest temperature explored (353 K).

Strong support in favor of the explanation given above comes from a parallel investigation regarding the rotation of guanine or adenine derivatives in platinum substrates in which the groups *cis* to the purine are H bond acceptors rather than H bond donors. In this case the trend is reversed and rotation is faster for adenine than guanine.

Factors Determining Conformer Distribution

The rotamer distribution found for the Me_3dienPtPen complex is, as expected, identical to that observed for Me_3-

dienPtEtG.[24] For the *syn*-(*R*,*S*) isomer, the preference is for the *endo* rotamer having the bulky six-membered ring on the same side of the platinum coordination plane as the *N*–Me groups (*endo*/*exo* ratio of 2 at 273 K). The *exo* conformation, which could form H bonds between the N–H group of *cis*-amines and O6 of the purine, is less favored. This evidence is contrary to a long-held belief that H bonds between purine derivatives and the NH group of platinum-carrier ligands are of primary importance in stabilizing the complexes in solution.[31] On the other hand, for the *rac*-Me_3dienPtPen isomer the *endo*/*exo* ratio is 1, as observed for the *rac* isomer of the complex with EtG. The *rac* isomer has one N–H and one *N*–Me group on each side of the platinum coordination plane; therefore, it is plausible that the two rotamers have similar stability.

In the Me_3dienPtdeoxy-Pen complex, the *syn*-(*R*,*S*) isomer again has a slight preference for the *endo* rotamer (*endo*/*exo* ratio of 1.3 at 273 K). Therefore, in this case also the *endo* rotamer, in which the six-membered ring of Pen is on the more crowded side of the platinum coordination plane, is more favored. Moreover, this *endo*/*exo* ratio of 1.3 is also observed for the *rac* isomer.

The *endo*/*exo* ratio, which is in the range of 2–2.5 for G derivatives (Pen and EtG complexes), is ca. 2.9 for the MeA complex (Table 1). In the case of MeA, the racemic isomer also shows a net preference for the *endo* rotamer (*endo*/*exo* ratio of ca. 2.2), while such a preference is much smaller for G complexes (*endo*/*exo* ratio in the range 1–1.2). Moreover, because for MeA the interconversion between rotamers is still very slow (on the NMR time scale) at the highest temperature explored, we do not know if the observed *endo*/*exo* ratio represents the thermodynamic equilibrium or is a kinetically determined composition.

In summary, at 298 K all complexes show a preference for the more crowded *endo* rotamer; moreover, the preference for such a rotamer appears to increase as the size of the substituent in position 6 of the purine ring system increases, independently of its charge. We do not have a good explanation for such a preference; however, we wish to point out that, in terms of free energy, the difference between the two rotamers is very small (less than 2.5 kJ·mol^{-1} at 300 K) and thus very difficult to account for.

For cases in which it has been possible, from kinetic data, to evaluate the difference in ΔH and ΔS between *endo* and *exo* rotamers (EtG, Pen, and deoxy-Pen complexes), the *endo* rotamer is lower in both enthalpy and entropy with respect to the *exo* rotamer (average 7 ± 2 kJ·mol^{-1} and 26 ± 4 J·K^{-1}·mol^{-1} for ΔH and ΔS, respectively). For the *syn*-(*R*,*S*)-Me_3dienPtEtG complex[24] we hypothesized that in the less abundant *exo* rotamer, interference between solvation shells of the purine C6 substituent and the *cis*-amine NH groups could lead to release of water molecules with a consequent slight increase in enthalpy and entropy. The stabilizing effect of the greater ΔS would not compensate for the destabilizing effect of the greater ΔH. The same explanation could also apply to the present case. We hope to gain a deeper insight in the *endo*/*exo* preference of rotamers by extending the investigation to purine bases carrying different substituents in position 8 of the purine ring system, so as to counterbalance the effect of substituents in position 6 of the purine.

Experimental Section

Materials: [Pt(Me_3dien)(NO_3)](NO_3) was prepared as previously reported.[22] Penciclovir [9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine, Pen] and 6-deoxy-Penciclovir (deoxy-Pen) were a gift of the Institute of Chemistry of Ljubljana, Slovenia. 9-Methyladenine (MeA) was prepared according to a literature method.[32]

Preparation of Adducts: Solutions of the adducts were prepared by treatment of [Pt(Me_3dien)(NO_3)](NO_3) (5 mM solution in D_2O at pH* = 1 or ca. 6) with a stoichiometric amount of L (L = Pen, deoxy-Pen, or MeA). Reactions were monitored by ^{1}H NMR until the disappearance of free L (or constant intensity ratio between free and complexed L in the case of an excess of L). Standard DNO_3 and NaOD solutions (in D_2O) were used to adjust the pH* of each sample directly in the NMR tube when necessary. 1D NMR studies were normally performed at 295 K. NOESY experiments were performed at 263 K (solvent D_2O/CD_3OD, 80:20 v/v) or 258 K (solvent D_2O/CD_3OD, 70:30 v/v).

1D NMR Spectroscopy: 1D NMR spectra were collected with a Varian Unity 600 spectrometer (spectral width, 6 kHz). The residual HOD peak was used as reference. In a typical experiment, a selective presaturation pulse (10 dB) was applied for 1 s to the residual HOD resonance. The FID was accumulated for 64 transients in blocks containing 16 K data points. Before Fourier transformation, the FIDs were baseline-corrected for DC offset before an exponential multiplication apodization function with a 0.2 Hz line broadening was applied. Selected ^{1}H chemical shifts of the purine base and the Me_3dien ligand are reported in Table 1.

2D NMR Spectroscopy: 2D NMR spectra (512×2048 matrices with a spectral window of 6–7 kHz in each dimension) were recorded with a Varian Unity 600 Spectrometer.[33] For the NOESY and ROESY experiments, a 500-ms mixing time was used. A 1-s presaturation pulse was typically used to saturate the HOD resonance. Spectra were processed using Felix 97.0 (MSI) with a Silicon Graphics INDY R4400 workstation. Typical processing involved zero filling of the t_1 dimension to 2048 points, exponential multiplication (0.4 Hz line broadening) in t_2, and applying a sine bell function shifted 90° over all points in t_1.

Line-Shape Analysis: For *syn*-(*R*,*S*)-Me_3dienPtL complexes (L = Pen or deoxy-Pen), the rate constants for interconversion between the *endo* and *exo* rotamers at different temperatures were calculated from line-shape analyses of ^{1}H NMR spectra collected with a Bruker AVANCE 300 MHz spectrometer. The simulation of the spectra was performed in the temperature range 255–353 K using DNMR6, a dynamic NMR spectra simulation FORTRAN code.[34] Rate constants for interconversion at different temperatures are reported in Table 2 and Table 3 for Pen and deoxy-Pen complexes, respectively. By applying the Eyring equation, the enthalpy and the entropy of activation ($\Delta H^{\neq}$ and $\Delta S^{\neq}$) were evaluated (Table 4).[35] In order to avoid ligand isomerization, a pH* value of 1 was used.

Supporting Information: See also footnote on the first page of this article. ln(k/T) as a function of 1/T for *syn*-(*R*,*S*)-Me_3dienPtPen at pH* = 1; selected regions of the ROESY spectrum (258 K, pH* = 5.3) for Me_3dienPtdeoxy-Pen; ln(k/T) as a function of 1/T for *syn*-(*R*,*S*)-Me_3dienPtdeoxy-Pen at pH* = 1; inversion recovery experiment for *syn*-(*R*,*S*)-Me_3dienPtMeA at pH* = 1.

Acknowledgments

This work was supported by the Ministero dell'Università e della Ricerca Scientifica e Tecnologica of Italy (MURST) Cofin. N. 2001053898 (to G. N.) and NIH Grant GM 29222 (to L. G. M.). The authors wish to thank the Institute of Chemistry of Ljubljana, Slovenia, for providing Penciclovir and 6-deoxy-Penciclovir, Dr. P. A. Marzilli for her valuable suggestions, Dr. N. G. Di Masi for NMR simulations, and Dr. F. Cannito for assistance in the manuscript preparation.

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Received: October 01, 2004

